

BEHAVIOURAL ECOLOGY OF FORAGING AND PREDATOR AVOIDANCE
TRADE-OFFS IN LAKE STURGEON (*ACIPENSER FULVESCENS*)

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ABSTRACT

I investigated Lake Sturgeon (*Acipenser fulvescens*) foraging and anti-predator behaviour. My goals were to understand: (1) The role of environmental change on foraging and anti-predator behaviour trade-offs. (2) The relative cost/benefit trade-off between escape behaviour and cover-seeking behaviour. (3) How development of several independent morphological traits affects anti-predator behaviours. I used simulated river mesocosms to study Lake Sturgeon behavioural ecology under controlled conditions. I found: (1) Foraging intensity was significantly higher during the night than the day as well as in turbid environments versus clear environments, indicating that decreased turbidity alone, may in part drive anti-predator behaviour and constrain foraging activity. (2) In high-risk clear-water environments, Lake Sturgeon responded to danger by evoking an escape response and seeking cover in rocky microhabitats. However, in low-risk turbid environments, Lake Sturgeon responded to danger by seeking cover in rocky microhabitats, but not fleeing to a significant degree. Cover-seeking behaviour may therefore be a relatively low-cost/high-benefit anti-predator strategy. (3) Strong evidence for trait co-dependence between escape responses and body size, where larger fish were able to elicit stronger escape responses. I also found that cover-seeking behaviour exhibited a complex multi-tiered relationship, representing a mixture of trait compensation and trait co-specialization that is dependent on specific combinations of morphological traits. These findings are important because they help us understand: (1) The degree to which anti-predator behaviour can be influenced by changing environmental conditions. (2) The relative cost/benefit trade-off between two common anti-predator behaviours. (3) How behaviour and morphology interact in species with a complex anti-predator phenotype.

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CHAPTER 1: INTRODUCTION

1.1 Lake Sturgeon (*Acipenser fulvescens*)

Taxonomy and systematics

The sturgeon family (Acipenseridae) first evolved in the Lower Jurassic period, approximately 200 million years ago (Bemis et al. 1997). It currently consists of 25 extant sturgeon species (19 species of Acipenserinae and 6 species of Scaphirhynchinae) which, along with two extant paddlefish species (*Polyodon spathula* and *Psephurus gladius*), comprise the order Acipenseriformes, the most numerous of all ‘living fossil’ fish (Birstein and DeSalle 1998). The largest sturgeon genus, *Acipenser*, is made up of 17 species, five of which are native to North America (Grande and Bemis 1996; Bemis and Kynard 1997; Findeis 1997; Scott and Crossman 1998). Of these, only Lake Sturgeon (*Acipenser fulvescens*) completes its lifecycle entirely in freshwater environments (Peterson et al. 2007).

Ecology and life-history

Lake Sturgeon spawn in the spring by scattering adhesive eggs over gravel and cobble substrates, allowing eggs to settle in the interstitial spaces between rocks which protects them from predators (Priegel and Wirth 1974; Becker 1983; LaHaye et al. 1992; Auer and Baker 2002; Bruch and Binkowski 2002). After hatching, yolk-sac larvae hide in the interstitial spaces of gravel and cobble substrates where they were spawned (Harkness and Dymond 1961; Wang et al. 1985; Kempinger 1988). Yolk-sac larvae are nourished entirely by their yolk-sac and do not feed exogenously. During the night larvae emerge from the substrate and drift downstream, settling within rocky interstitial spaces once again at daybreak (Kempinger 1988; LaHaye et al.

1992). After approximately two weeks the yolk-sac is entirely consumed, marking the transition to exogenous feeding. At this point, sturgeon must leave rocky interstitial spaces to forage and therefore become more susceptible to predation. Larval sturgeon are thought to feed primarily on benthic invertebrates, including crustaceans, insect larvae, mollusks, and isopods (Harkness and Dymond 1961; Wallus 1990; Chiasson et al. 1997). Several morphological adaptations begin to develop at this stage, presumably serving an anti-predator function.

Morphology

Lake Sturgeon undergo a dramatic morphological change in a relatively short time period. The extent and rate at which these morphological changes proceed is so dramatic it is almost like metamorphosis (Harkness and Dymond 1961; Peterson et al. 2007). Notable features of sturgeon morphology include five rows of scutes (armour/spines), a mottled (cryptic) body pattern that develops in juveniles, and fast-growing bodies in early life-history stages.

A series of protective armoured plates (scutes) are prominent in juvenile Lake Sturgeon (Peterson et al. 2007) and may act to reduce their vulnerability to predation due to the severe consequence of handling or capturing armoured prey (Brodie and Brodie 1990). Scutes are generally absent in White Sturgeon (*Acipenser transmontanus*) until they reach about 25 mm (Gadomski and Parsley 2005a), and develop at comparable ages in Lake Sturgeon. Scute growth differs between species and likely relates to predation. In Lake Sturgeon, scutes are resorbed when they outgrow local predators, whereas sturgeon species that spend at least part of their lives at sea (anadromous), where predators can grow to be much larger, retain their scutes (Peterson et al. 2007). Scute retention, however, is costly. Since scutes reduce streamlining, additional energy must be allotted to locomotion (Peterson et al. 2007). Due to the anti-predator function of scutes,

they may compensate for anti-predator behaviour by allowing sturgeon to forage more than sturgeon without scutes.

Mottled (cryptic) body colouration in Lake Sturgeon, as in other animals, probably serves an anti-predator function. Changes in colour pattern during the transition between larval and juvenile stages are among the most noticeable morphological changes (Vladykov and Greeley 1963; Priegel and Wirth 1971; Peterson et al. 2003). Larval sturgeon have a simple striped colouration, but mottling develops in older (> 4 month old) fish. This cryptic mottled colour pattern likely makes sturgeon less detectable to visual predators.

Lake Sturgeon allocate a disproportionate amount of energy to somatic growth during the juvenile stage (Beamish et al. 1996; LeBreton and Beamish 2004). Since predation risk is largely size dependent, this strategy may help reduce mortality during the most vulnerable early life stages (Peterson et al. 2007).

Predator-prey interactions

Predators are often responsible for influencing prey behaviour, morphology and physiology (Sih et al. 1985) and understanding Lake Sturgeon anti-predator behaviour is essential for our knowledge of the ecology of this species. Furthermore, it is fundamental to understand the dynamics that shape predator-prey interactions in young fish as predation may be the single most important factor contributing to fish mortality in early life stages (Hunter 1976). Selection on anti-predator behaviour should therefore be particularly strong at this life stage. Remarkably, to my knowledge, no studies have experimentally investigated predator-prey interactions in Lake Sturgeon although vulnerability to predators has been examined in White Sturgeon (Gadomski and Parsley 2005a,b,c) and Pallid Sturgeon (*Scaphirhynchus albus*) (French

et al. 2010). Surprisingly, Hintz et al. (2012) were the first to document predator-avoidance behaviour in any sturgeon species, demonstrating that Shovelnose Sturgeon (*Scaphirhynchus platyrhynchus*) tended to avoid areas with predators by moving to safe areas and would flee from an attacking predator.

Conservation status

Lake Sturgeon species were once common and widespread in Canadian rivers but anthropogenic stressors such as habitat degradation and overfishing are thought to be primarily responsible for their precipitous decline (Williams et al. 1989; Rochard et al. 1990; Boreman 1997; Bogue 2000; Auer 2004). All sturgeon species are now listed as endangered in part of their ranges, and most in all of their ranges (Birstein et al. 1997; Billard and Lecointre 2001). Additionally, at the time of this writing, sturgeon are more critically endangered than any other group of species on the planet (IUCN 2010). Their unique life history and declining populations have led to a renewed interest in sturgeon biology. Improved basic knowledge of Lake Sturgeon ecology and biology has recently emerged (reviewed in Peterson et al. 2007) but is still far from complete.

1.2 Objectives

Sturgeon anti-predator behaviour in general, and Lake Sturgeon anti-predator behaviour in particular, is not well understood. Unique sturgeon life-history, anti-predator behaviour, and morphology collectively provide a rare opportunity to answer basic questions about behavioural ecology of predator-prey interactions that are not possible with most model organisms.

In Chapter 2, I manipulated water turbidity (water cloudiness or haziness), an important feature in aquatic environments that can play a large role in mediating predator-prey interactions. Sturgeon are not visual foragers, a simple fact that enabled me to investigate how changes in turbidity can alter anti-predator investment (inactivity to avoid detection) and reduce foraging behaviour even in the absence of predators. The degree to which environmental variables alone can elicit or relax anti-predator behaviour is presently not well understood.

In Chapter 3, I investigated the cost/benefit trade-off of two common anti-predator behaviours: escape behaviour and cover-seeking behaviour. My goal was to evaluate whether escape or cover-seeking behaviours would be abandoned sooner in low-risk (turbid) environments. Understanding how each behaviour changes across levels of risk may give us some indication of the relative cost/benefit trade-off of each of these two behaviours which are common and widespread in the animal kingdom.

In Chapter 4, I evaluated how Lake Sturgeon morphology co-varies with anti-predator behaviour. Sturgeon are unique in that they possess several independent anti-predator morphological adaptations (1) scutes (armour/spines), (2) a mottled (cryptic) body pattern, and (3) fast-growing bodies, that undergo dramatic changes in a relatively short time period. My goal was to understand how anti-predator behaviours vary in response to changes in morphology.

1.3 Anticipated Significance

My research is aimed at understanding fundamental questions about (1) the role of environmental change on anti-predator behaviour, (2) the relative cost/benefit trade-off between escape behaviour and cover-seeking behaviour, and (3) how development of several independent morphological traits affects anti-predator behaviours. Furthermore, as it is becoming increasingly

important for behavioural ecologists to design studies that not only answer fundamental questions, but also provide insights for conservation of endangered species and their habitats (Caro and Sherman 2011), much of my research also has a direct conservation component. Specifically, Chapter 2 is concerned with how changing environmental conditions can influence foraging patterns, with specific implications to changes in turbidity in sturgeon habitats. Likewise, Chapter 3 is concerned with the role that structural habitat heterogeneity, in this case, rocky microhabitats, plays in allowing sturgeon to elicit low-cost/high-benefit anti-predator behaviours. Together, these two studies should help us understand how changes in turbidity and structural habitat heterogeneity affect sturgeon anti-predator behaviour. Furthermore, environmental changes and habitat alterations are omnipresent across the planet, and understanding the role they play in predator-prey dynamics is important for all animal species because nothing reduces individual fitness more than being consumed by a predator.

1.4 Thesis Format

This thesis has been organized in manuscript format for publication. As a result, there may be some repetition of information throughout the text.

Chapter 2 has been submitted to *Behaviour*, under the joint authorship of Annessa B Musgrove, Douglas P Chivers and Maud CO Ferrari (University of Saskatchewan).

Chapter 3 has been submitted to *Ethology*, under the joint authorship of Douglas P Chivers and Maud CO Ferrari (University of Saskatchewan).

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CHAPTER 2: RISK IN A CHANGING WORLD: ENVIRONMENTAL CUES DRIVE ANTI-PREDATOR BEHAVIOUR IN LAKE STURGEON (*ACIPENSER FULVESCENS*) IN THE ABSENCE OF PREDATORS

2.1 Introduction

Human-induced rapid environmental change is putting animals in evolutionarily novel situations to which they may or may not be adapted. A recent meta-analysis of more than 3000 rates of phenotypic change suggests that most of the responses of animals to rapid environmental change involve phenotypic plasticity rather than immediate genetic evolution (Hendry et al. 2008). In the face of such challenges, animals often display alterations in behaviour, as it is one of the fastest phenotypic changes, and because behaviour can usually be much more plastic than other phenotypic traits.

Behavioural plasticity refers to an animal's ability to vary its behaviour according to different conditions (West-Eberhard 1989) and may be the quickest way animals adjust to a changing environment. For example, lizards subjected to high temperatures will alter their basking behaviour to regulate their physiological performance (Huey et al. 2003). Similarly, changes to predator abundance or performance can have a tremendous impact on behavioural decisions regarding foraging intensity or reproductive investment (Sih 1987; Lima 1998; Stankowich and Blumstein 2005). Behavioural plasticity can therefore be invaluable for populations subjected to rapidly changing climates and habitats. Alternatively, in a rapidly altered environment, a formerly adaptive behaviour may become non-adaptive and have fitness consequences. Environmental cues that have been correlated with predator abundance or performance over evolutionary history may have significant effects on present-day prey

behaviour even in the absence of predators. However, how changing environmental conditions influence behaviourally plastic traits is not well understood (Ghalambor et al. 2010).

Organisms inhabiting aquatic ecosystems are ideal for testing the fitness consequences of behaviour in response to environmental changes. Aquatic ecosystems have been subjected to a wide range of environmental changes including changes to temperature, decreased pH, contamination and habitat fragmentation (Schindler 1988; Winder and Schindler 2004; Orr et al. 2005). Moreover, there is increasing concern that anthropogenic activities are leading to changes in turbidity (Chivers et al. 2013). Turbidity (water haziness or cloudiness) results in benthic smothering, changes rates of photosynthesis, and can often result in substantial changes to community structure (Liljendahl-Nurminen et al. 2008). River turbidity levels are rapidly changing worldwide (Kundzewicz et al. 2008) and in some areas river turbidity fluctuates with water-flow regimes, where turbidity levels are linked to flow rates (Dewson et al. 2007). Decreases in turbidity can be attributed to both biotic agents, such as changes in faunal community structure (Skubinna et al. 1995) or anthropogenic factors, such as dam construction along large rivers (Johnson et al. 1995). Predator-prey interactions, in particular, are influenced by turbidity levels (Bonner and Wilde 2002; Chivers et al. 2013).

Turbidity decreased foraging success for predatory Banded Kokopu (*Galaxias fasciatus*), Common Galaxias (*G. maculatus*) and Sablefish (*Anoplopoma fimbria*) (Rowe and Dean 1998; De Robertis et al. 2003), and impeded learned predator recognition in Fathead Minnows (*Pimephales promelas*) (Ferrari et al. 2010; Chivers et al. 2013). However, higher turbidity also reduced predation risk for Northern Pike (*Esox lucius*) (Lehtiniemi et al. 2005), White Sturgeon (*Acipenser transmontanus*) (Gadomski and Parsley 2005b), damselflies (*Ischnura elegans*) (Van de Meutter et al. 2005), and allowed Fathead Minnows to use feeding areas occupied by a

predator (Abrahams and Kattenfeld 1997; Chiu and Abrahams 2010). Furthermore, Ferrari et al. (2014) found that Delta Smelt (*Hypomesus transpacificus*) that evolved in turbid-water environments are decimated by predators in clear-water environments as smelt rely exclusively on turbidity to avoid predators. Turbidity can therefore either have a positive or negative impact on a species, depending on their particular life-history, their level of exposure to visually-guided predators, and the environmental conditions in which they evolved.

In the present study, I looked at the effect of turbidity on foraging activity in Lake Sturgeon (*Acipenser fulvescens*) in the absence of predators to investigate the degree to which environmental cues alone can affect foraging activity. Nocturnal foraging may reflect a balance between foraging and anti-predator behaviours. However, due to the severe cost of failing to avoid a predator, this balance may be shifted away from active foraging and toward anti-predator inactivity. Thus, given the opportunity, nocturnal animals may forage actively during the day under the cover of turbidity. I predict that foraging activity will be lowest during the day, and highest during the night, owing to the increased risk of predation by visual predators during the day. Second, I predict that due to the anti-predator benefit of turbidity, foraging activity levels will be higher in turbid environments than in clear-water environments. Third, I predict that as a result of growth and development, the difference between clear- and turbid-water foraging activity will be greater in young fish than in older fish.

Sturgeon are particularly good candidates to investigate the role of turbidity on anti-predator behaviour because sturgeon are a non-visual feeding fish (Loew and Sillman 1993; Boglione et al. 1997; Rodríguez and Gisbert 2002). Sturgeon forage primarily by using four sensory barbells on the ventral side of their body immediately anterior to the mouth (Peterson et al. 2007). These barbells decode the chemical composition of potential prey items, allowing them

to forage without the use of sight. Furthermore, sturgeon electrosense has recently been described, whereby sturgeon can detect the electrical signals of potential prey items, allowing sturgeon to recover prey hidden beneath sandy substrates and forage effectively in turbid rivers and lakes (Zhang et al. 2012). Thus, while turbidity may impair the ability of visual predators to detect them, it does not detrimentally influence sturgeon foraging ability. In the wild, most fish spend part of their day in an active foraging state, and the other half in an inactive state that is intimately linked with predator avoidance (Helfman 1993). This is especially true of juvenile sturgeon, which forage actively during the night and reduce activity during the day to avoid predators (Richmond and Kynard 1995). Turbidity may provide cover from visually-guided predators, allowing sturgeon to increase foraging activity in turbid environments relative to clear-water environments. Therefore, even in the absence of predators, water clarity levels may exert strong effects on sturgeon foraging and anti-predator behaviours.

2.2 Materials and Methods

Study species

Lake Sturgeon were obtained from Sustainable Sturgeon Culture, Ontario, in May 2012 where they were spawned from wild broodstock endemic to the Rainy River, Ontario. They were housed at the RJF Smith Centre for Aquatic Ecology, held in a 640-L flow-through tank, and fed every 6 h with live brine shrimp (*Artemia* spp.) and crushed frozen bloodworm (Chironomidae spp.). They were maintained in dechlorinated tap water between 16-18 °C under a 14:10 hour artificial light:dark cycle. However, the RJF Smith Centre for Aquatic Ecology is designed in such a way that sunlight illuminates the facility. Thus, a 17:7 natural light:dark cycle (resembling local light-patterns) predominated during the course of the study.

Experimental set-up

Trials took place in 100 L polyurethane D-ended simulated river mesocosms measuring 1 m long x 50 cm wide x 30 cm high (Figure 2.1).

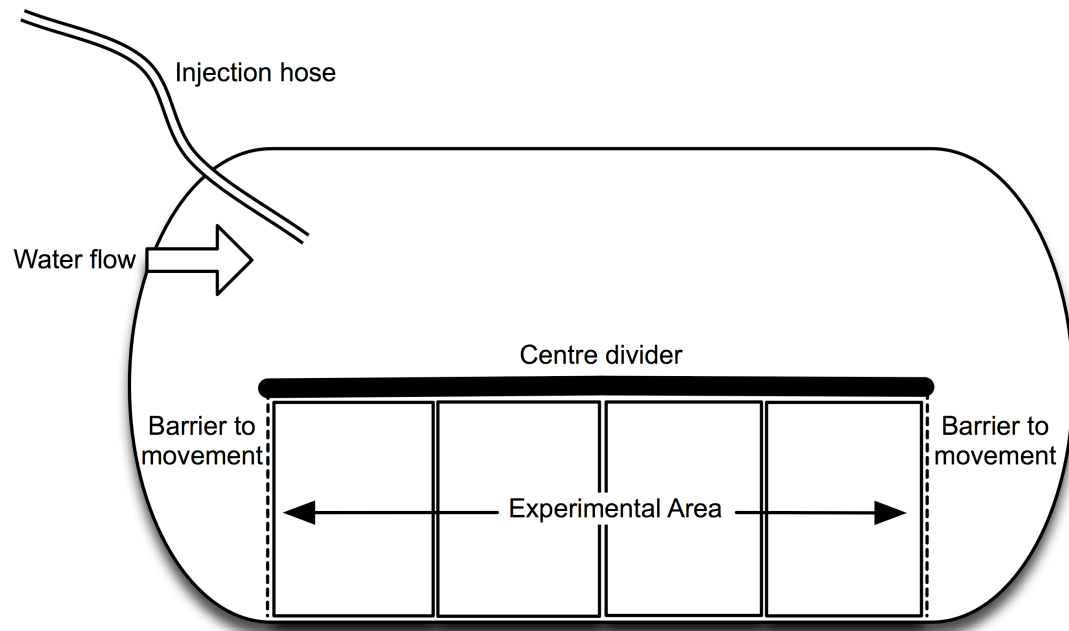


Figure 2.1 Overhead view of mesocosms used to simulate river microhabitats during experiments.

Each mesocosm was divided in half longitudinally with an opaque acrylic panel measuring 60 cm long to allow for the circular flow of water with a submersible $15 \text{ L} \cdot \text{min}^{-1}$ powerhead that provided a flow of $2\text{-}3 \text{ cm} \cdot \text{sec}^{-1}$. A rectangular section of the long-edge of the mesocosm measuring 55 cm long x 20 cm wide was segregated using vinyl-coated fiberglass mesh mounted on PVC frames. Inside the mesocosm four boxes were placed, each measuring 20 cm long x 13.5 cm wide x 1.5 cm deep, which held substrates used to simulate river microhabitats. A rocky microhabitat was created by layering cobble (grain size: 10-20 mm) under rocks (grain size: 30-70 mm) stacked to a peak 5 cm high. A sandy microhabitat was

created by filling a substrate box with sand (grain size: 0.25–1.2 mm) 1.5 cm deep. Two boxes of each microhabitat type were randomly arranged in the mesocosm before fish were introduced. The rectangular section of the mesocosm containing four microhabitat boxes, two of each type, between the mesh barriers, comprised the experimental area. Each mesocosm was filled with 25 L of water to a height of 5.5 cm over the substrate boxes to control for use of the water column by the fish. Water was either left clear or turbidity was adjusted by adding 2.25 g of bentonite (an inert colloidal clay, Sigma-Aldrich; CAS number 1302-78-9) immediately before the fish were added producing a concentration of $0.09 \text{ g} \cdot \text{L}^{-1}$, ~ 22.5 NTU (Nephelometric Turbidity Units), equivalent to ~ 17 cm secchi depth (Shoup and Wahl 2009). Turbidity measurements were taken using a LaMotte 2020e portable turbidity meter (LaMotte Company, Chestertown MD); secchi depth measurements were taken using a 120 cm turbidity tube for intermediate turbidity levels (Wildlife Supply Company, Yulee FL). Each mesocosm was reconditioned with 1.12 g of bentonite every 6 h to account for loss of suspended clay with time yielding an NTU range of 18.2 - 27.8 and a secchi depth range of 20 cm - 15 cm, respectively, during the experiment. In clear-water mesocosms, NTU was equal to 0.1. Thus, the clear- and turbid-water mesocosms provided very different physical environments for the fish during the course of the study.

Experimental protocol

I investigated how turbidity (clear vs. turbid environments), time-of-day (day vs. night) and age-class (early vs. late) influenced diurnal foraging activity patterns. Early age-class fish were between 42 and 54 days post-hatch (dph) (between 28 and 40 days after the onset of exogenous feeding); late age-class fish were between 57 and 69 dph (between 43 and 55 days after the onset of exogenous feeding). These two age-classes were selected in order to evaluate if

any effects due to turbidity or time-of-day were weaker in older fish, since a previous study (Peake 1999) found that young Lake Sturgeon were more active during the night relative to older conspecifics. Four combinations resulted (early age-class/clear, early age-class/turbid, late age-class/clear, and late age-class/turbid), and 20 replicates were run per treatment combination. Prior to the onset of trials three fish were arbitrarily selected from the holding tank, placed in the experimental area of each mesocosm to provide an ecologically relevant social context, and left to acclimate for 24 hours. During the night, an incident light at an approximate intensity of 0.25 lux (equivalent to the light of a full moon on a clear night) provided some illumination. Trials were conducted during June and July 2012 by using overhead cameras mounted with infrared lights to record both diurnal and nocturnal sturgeon behaviour over a 24 hour period. All fish were fed every 6 hours during the acclimation and observation periods to control for food-driven changes in activity. At the completion of trials, fish were placed in a separate holding tank and not used for subsequent trials to maintain statistical independence among trials.

Video analysis

Foraging activity was scored as the number of times a randomly-selected focal fish passed over a superimposed 10 x 6.75 cm grid during a 3-minute interval at the start of each hour for 24 hours. The side of each square was equivalent to approximately 3 to 5 body lengths of the fish. Since sturgeon forage by actively cruising along the substrate surface, activity level is a good proxy of foraging activity, which includes feeding, moving between foraging patches, and exploring their environment to seek out resources.

Statistical analysis

Data were analyzed using the R environment for statistical programming (version 2.15.2; R Development Core Team 2012). Due to a loss of video files from two late age-class /clear-water mesocosms (48 3-minute intervals) I based the analysis on the remaining 480 early age-class / clear-water, 480 early age-class / turbid-water, 432 late age-class / clear-water, and 480 late age-class / turbid-water behavioural observations, comprising a total of 1872 3-minute observation intervals. Each mesocosm was considered the sampling unit. All statistical analyses were run using raw values; however, plots and reported results of activity level are presented in units per hour. Values are mean \pm 1 standard error.

To quantify foraging activity through time within each environment, and to compare foraging activity through time between environments, polynomial regression models were fit to mean hourly activity values for each treatment (n = 40 replicates/hour in turbid environments; n = 38 replicates/hour in clear environments). This was accomplished by (1) identifying an initial model that described the data well (i.e., contained only significant terms), (2) deriving best-fit parameter estimates for two separate models (one for each treatment) based on the number of parameters in the initial model, and (3) systematically combining coefficients to identify the most parsimonious set of model parameters. This simplified parameter set would provide a better balance between model accuracy and complexity (determined using Akaike's Information Criterion with a correction for small sample sizes [AICc], where models with lower AICc values were considered more parsimonious; Burnham and Anderson 2002).

I tested the effects of time-of-day, age-class, and treatment on foraging activity using a 3-way ANOVA with mesocosm as a nested variable to control for spatial autocorrelation within each mesocosm. Due to a high proportion of zeros in the dataset (~15 %, as a result of the absence of foraging activity at certain times) I ran the analysis on values of average activity level during either day or night in each mesocosm. Model residuals satisfied assumptions of normality and homoscedasticity, no data points had undue leverage, and no outliers were identified.

2.3 Results

A single quadratic model was initially fit to combined clear- and turbid-water mean activity data since all terms in this model were significant, whereas lower- and higher-order models both contained non-significant terms (Table 2.1).

Table 2.1 Model parameter estimates for the combined dataset relating Lake Sturgeon foraging activity to time of day.

		Estimate	Std. Error	t value	P value
Linear model	a	579.138	64.35	9	< 0.001
	b	-5.528	4.794	-1.153	0.255
Quadratic model	a	930.038	56.881	16.35	< 0.001
	b	-101.228	11.456	-8.836	< 0.001
	c	4.161	0.481	8.65	< 0.001
Cubic Model	a	826.11026	66.73124	12.38	< 0.001
	b	-40.45162	25.67195	-1.576	0.1223
	c	-2.58767	2.62635	-0.985	0.3299
	d	0.19561	0.07499	2.609	0.0124

Parameter estimates are the values of the coefficients that define each curve.

Two quadratic models were subsequently fit, one for each treatment, and all terms in each separate model were significant (Table 2.2).

Table 2.2 Quadratic model parameter estimates relating Lake Sturgeon foraging activity to clear and turbid treatments.

		Estimate	Std. Error	t value	P value
Clear water	a1	904.645	37.007	24.45	< 0.001
	b1	-100.924	7.453	-13.54	< 0.001
	c1	3.837	0.313	12.26	< 0.001
Turbid water	a2	1072.1317	44.7664	23.949	< 0.001
	b2	-95.2706	9.0158	-10.567	< 0.001
	c2	3.4373	0.3786	9.079	< 0.001

Term definitions: a = y-intercept; b = vertex position; c = rate of change.

Combining coefficients b: vertex position (time of minimum activity level), and c: rate of change (speed of increase and decrease in activity level) yielded the most parsimonious set of models (Table 2.3). Coefficient a: y-intercept (maximum activity level), however, was not combined. The top model had a model weight of 0.52 which can be interpreted as meaning there is a 52% chance that it is the best model describing the data (Symonds and Moussalli 2011). Furthermore, all three top models had a combined model weight of 0.92, indicating that combining vertex position and rate of change, but not maximum activity level (y-intercept), yields a strong model fit.

Table 2.3. AICc values for models sharing common terms.

Model adjustments	<i>k</i>	AICc	Δ AICc	w_i
Combined <i>b</i> and <i>c</i> terms	5	600.79	0	0.52
Combined <i>b</i> term	6	602.68	1.89	0.20
Combined <i>c</i> term	6	602.75	1.96	0.20
None (no combined terms)	7	605.42	4.64	0.05
Combined <i>a</i>	6	606.89	6.10	0.02
Combined <i>a</i> and <i>c</i> terms	5	609.79	9.00	0.01
Combined <i>a</i> and <i>b</i> terms	5	613.89	13.10	0.00
All combined terms (single model)	4	618.12	17.33	0.00

Term definitions: a = y-intercept; b = vertex position; c = rate of change. *k* = number of parameters in the model. w_i = model weight.

These results indicate that in both clear- and turbid-water treatments, foraging activity decreased to a minimum at the same time-of-day (around mid-day), and rate of increase and decrease in foraging activity was similar between the two treatments. Therefore, fish in turbid environments were consistently more active during both the day and at night (Figure 2.2).

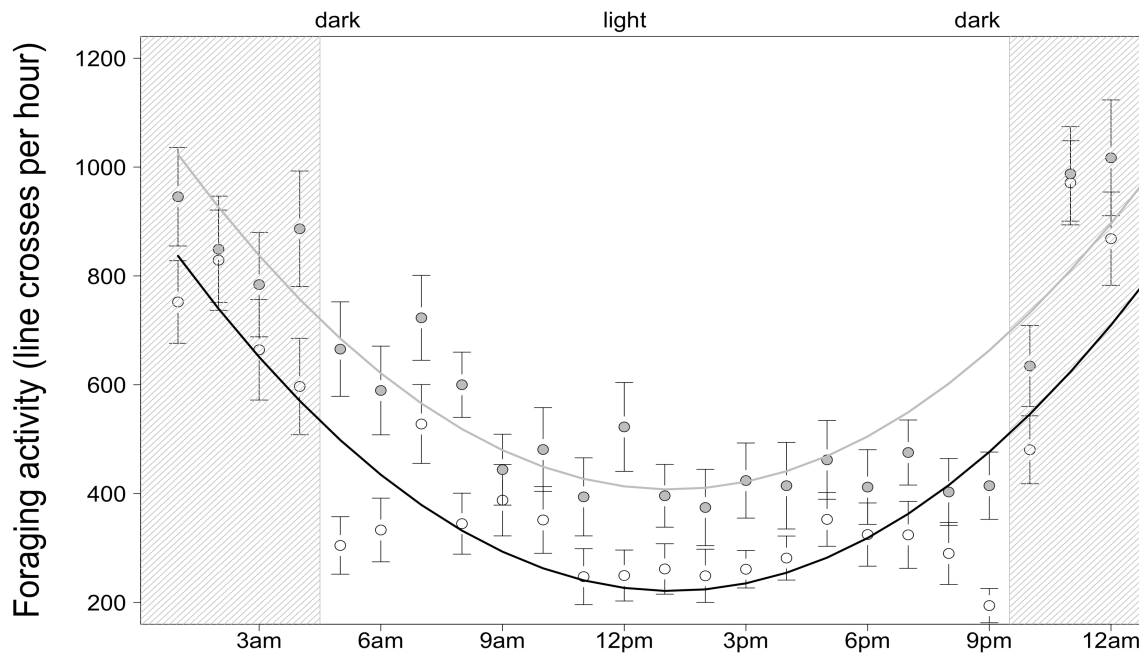


Figure 2.2 Mean (± 1 SE) foraging activity for each hour in either clear $n = 38$ (white points) or turbid $n = 40$ (grey points) environments. Polynomial models are fit to either clear- (black line) or turbid-water (grey line) mean activity values (see text for details).

Foraging activity was significantly influenced by the main effects of turbidity and time of day (Figure 2.3; Table 2.4).

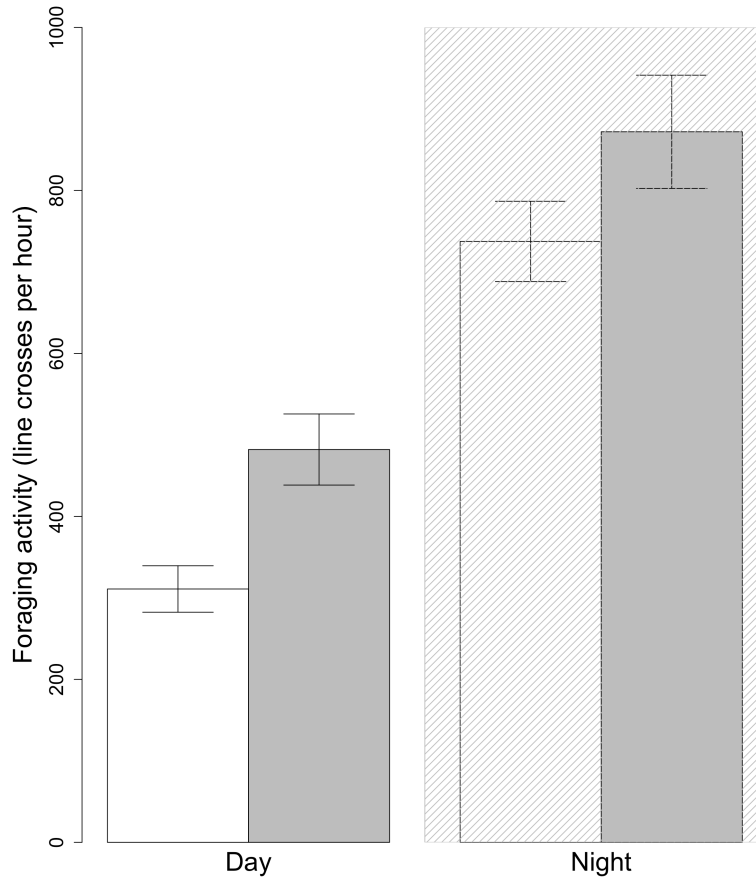


Figure 2.3 Mean (± 1 SE) foraging activity during the day (unshaded region) and during the night (shaded region) in clear environments (white bars) or turbid environments (grey bars). Clear-water/day activity $n = 38$; turbid-water/day activity $n = 40$; clear-water/night activity $n = 38$; turbid-water/night activity $n = 40$.

Table 2.4 ANOVA table showing the effect of time of day (day vs. night), age (early-stage vs. late-stage), and turbidity (clear vs. turbid) on foraging activity.

Source		<i>df</i>	<i>F</i>	<i>P</i> value
Between subjects	Turbidity	1, 74	5.849	0.018
	Age	1, 74	0.026	0.872
	Turbidity * Age	1, 74	0.812	0.370
Within subjects	Time of day	1, 74	148.173	< 0.001
	Turbidity * Time of day	1, 74	0.298	0.587
	Age * Time of day	1, 74	3.572	0.063
	Turbidity * Age * Time of day	1, 74	0.185	0.669

Bold type indicates significant *P*-values at a 0.05 alpha level.

No significant interactions were identified, but the interaction between age-class and time of day approached significance.

2.4 Discussion

Foraging activity was significantly higher in turbid environments versus clear environments, indicating that turbidity may drive foraging activity. Foraging activity was consistently higher in turbid environments during the night and the day, and (while not captured by the model) activity level in clear water decreased abruptly in the presence of light and increased sharply in the absence of light, whereas activity level in turbid water changed more gradually in both cases (Figure 2.2). These results indicate that foraging activity may be driven by available light, where foraging is immediately abandoned at the first sign of light in the early morning and immediately resumed when light levels drop in the evening. Fish behaviour has long been known to be dramatically affected by the diel cycle, where light levels are negatively correlated with foraging activity and positively correlated with anti-predator behaviour (Helfman 1993). However, turbidity reduced this effect, likely due to the increased attenuation it imposes on visible light decreasing its penetration depth and intensity. These results demonstrate that simple changes in environmental conditions which are linked to predator performance can exert strong effects on prey fish behaviour even in the absence of predators.

Fish in turbid environments prolonged foraging activity suggesting that young sturgeon may forage at maximum anti-predator effort (i.e., forage with a bias toward safety) (Lima and Bednekoff 1999). If this is the case, then turbidity may allow young sturgeon to forage beyond their minimal caloric requirement, accelerating somatic growth and the rate of development of morphological defenses, further reducing the need for behavioural anti-predator effort. Consistent with this line of reasoning, I found that the interaction between time of day and age trended toward significance, suggesting that as sturgeon grew older the diurnal foraging pattern

observed became less pronounced. It may be that increased somatic growth, defensive morphological development, or some combination of both may compensate for anti-predator behaviour in this species (i.e., trait compensation) (Dewitt et al. 1999). Another possibility is that increased nutritional requirements of older fish are responsible for this change in behaviour. Over a longer period of time the differences in foraging activity between day and night may become increasingly attenuated.

Evolution of behaviourally plastic foraging behaviour

Whether or not an animal can elicit a plastic response can give some indication as to the ecological constraints that have shaped its behaviour. Adaptive evolution of environmentally-mediated behaviourally plastic responses relies on four elements: (1) environments must exhibit variability; (2) fitness must be driven by the match between a behaviour and the current environment; (3) individuals should be able to elicit an appropriate behavioural change based on a reliable cue; and (4) no single behaviour should be useful in all environments (Via and Lande 1985; Moran 1992). Since behavioural plasticity tends to be favoured in variable environments where the cues are reliable, then it follows that the environmental agent evoking a plastic response should reliably influence predation, reproduction or some other component of fitness. On the other hand, where behaviourally plastic responses are lacking, we may expect the environment is invariable, or that variation in the environment does not reliably predict a fitness-critical outcome. In the present study, the evidence that turbidity alone can drive foraging activity suggests that turbidity may have been a reliable cue of decreased predation risk during sturgeon evolutionary history. However, if predators are absent, decreased foraging in clear-environments becomes increasingly maladaptive (i.e., may be an evolutionary trap) if reduces

survival or reproductive success. As ecosystems continue to change, understanding how environmental cues interact with predation risk will be important in order to help predict the effect of environmental changes on behaviours shaped and driven by predation.

Foraging, predation, and environmental change

While foraging and avoiding predation are both necessary for survival, strategies shaped by predation are often under especially strong selection owing to the high (asymmetrical) costs of losing one's life. This high cost often shifts the balance toward conservative (safe) strategies that drive the probability of failure (death) toward zero (Bouskila and Blumstein 1992). Failing to feed in any one particular instance may not result in death, but failing to avoid a predator can be disastrous for prey. This idea is that of the 'life-dinner principle' coined by Dawkins and Krebs (1979), and is generally believed to manifest itself in predator-prey systems (but see Brodie and Brodie 1999). Given the severe costs of failing to avoid a predator, it seems reasonable that environmental variables that reliably influence predation are used as cues to drive anti-predator behaviour. In the present study this may be the case. Here, the risk of predation by visual predators may be reduced in turbid environments relative to clear-water environments. Other environmental variables, such as temperature, salinity, or pH that are associated with predator performance, may have similar effects in other systems. Future work exploring the interconnectedness of environmental variables and behaviourally plastic anti-predator responses will help us better understand the suite of effects changing environments have on animal behaviour.

Sturgeon conservation in natural environments

It is becoming increasingly important for researchers to design studies that not only answer fundamental scientific questions, but also provide insights for conservation, protection, and management of study systems and habitats (Caro and Sherman 2011). All sturgeon species are now listed as endangered in part of their ranges, and most in all of their ranges (Billard and Lecointre 2001). Behavioural plasticity may be especially important for sturgeon due to their relatively late age at maturity, long generation times, and protracted spawning periodicity (Peterson et al. 2007). Moreover, the rate of molecular evolution in Acipenseriformes has been found to be nearly half as fast as teleost fish (Krieger and Fuerst 2002). In endangered sturgeon populations with little genetic diversity or low population growth, adaptive evolution becomes increasingly unlikely (Carroll and Watters 2008; Hendry et al. 2011), possibly accelerating the role that behavioural plasticity plays in mediating behavioural responses to changing environments. Changing turbidity levels in the aquatic environment may influence sturgeon behaviour and may have fitness consequences. Turbidity, therefore, should be considered in habitat restoration programs. Increased turbidity may be beneficial to young sturgeon, whether predators are present or not. Clear water, on the other hand, may significantly restrict foraging activity in Lake Sturgeon, even in the absence of predators.

Conclusions

These results describe how environmental conditions alone can influence anti-predator behaviour, thereby driving foraging activity and potentially affecting fitness. Continuing to uncover the role of environmental variables on behaviourally plastic traits is essential for understanding the processes that gave rise to present-day foraging strategies. Even now the

degree to which behaviourally plastic responses are influenced by changing environmental cues is not well understood. Furthermore, given that rapidly changing climates and habitats represent important challenges faced by the majority of animal species on the planet, and are leading to rapid declines in global biodiversity, they deserve more attention.

CHAPTER 3: RELATIVE COST/BENEFIT TRADE-OFF BETWEEN COVER-SEEKING AND ESCAPE BEHAVIOUR IN AN ANCESTRAL FISH: THE IMPORTANCE OF STRUCTURAL HABITAT HETEROGENEITY

3.1 Introduction

Predation is a major selective force contributing to morphological, behavioural and physiological adaptations in prey (Sih et al. 1985; Stankowich and Blumstein 2005). Behavioural responses, however, are unique in that they allow prey to instantaneously adjust their actions to influence the outcome of an encounter with a predator (Helfman 1986, 1989; Lima and Dill 1990). Anti-predator behaviours are incredibly diverse—a result of tremendous variation in selective pressures by predators on prey. Nevertheless, two anti-predator behaviours are common and widespread in the animal kingdom: fleeing from predators and seeking cover in protective habitats.

Fleeing can occur before an attack (effective avoidance) or after an attack (escape) (Lima and Dill 1990). In the former case, prey must make choices regarding if and when to flee and the magnitude of the response. Fleeing unnecessarily, or in an exaggerated way, reduces the amount of time an animal can forage (Lima and Dill 1990). Therefore, if prey are selected to maximize fitness during predator-prey encounters, an optimal response can be thought of as being scaled appropriately to the level of risk (Puttlitz et al. 1999; Cooper and Frederick 2007). Protective habitats can be defined as any physical environment that reduces the danger posed by a predator. These habitats are usually separate from foraging locations, and retreat into protective habitat can provide protection at the cost of feeding (Ydenberg and Dill 1986). Similar to fleeing behaviour,

described above, decisions concerning when to retreat to protective habitats and the amount of time spent there, should be scaled to the level of perceived risk (Křivan 1998).

Escape behaviour and cover-seeking behaviour in response to predators have been investigated independently (escape behaviour: Abrahams 1995; Hilton et al. 1999; Stankowich and Coss 2005; cover-seeking: Werner et al. 1983; Gilliam and Fraser 1987; Gregory and Levings 1996; Snickars et al. 2004) and together (Kiesecker et al. 1996; Stankowich and Coss 2007; Cooper 2009). However, while the cost of these different behaviours have been given theoretical consideration (Křivan 1998), to my knowledge they have not been examined empirically. Lake Sturgeon (*Acipenser fulvescens*) provide an excellent opportunity to investigate the relative cost/benefit trade-off between escape and cover-seeking behaviours since they respond to risk by performing both behaviours simultaneously.

In the present study, I investigate escape responses (fleeing) and movement into rocky microhabitats (cover-seeking) in response to predation risk in environments having or lacking turbidity (which reduces the level of perceived risk for sturgeon). Based on preliminary trials I found that in high-risk (clear) environments both responses were elicited together in the presence of danger (alarm cues). However, I predict that in low-risk (turbid) environments, there would be three possible outcomes. First, if I find no difference in escape response and cover-seeking in the turbid-water environment relative to the clear-water environment, then both strategies may be equally costly and/or beneficial behaviours employed even in low-risk encounters with a predator. However, if the magnitude of cover-seeking is reduced in turbid environments, while escape responses remains unchanged, then cover-seeking may be regarded more costly or less beneficial and avoided when risk is reduced. Conversely, if the magnitude of escape response is reduced in low-risk environments while cover-seeking remains unchanged, then escape

behaviour may be regarded as more costly or less beneficial than cover-seeking behaviour and avoided whenever possible. I based my hypotheses and predictions on the theoretical basis that (1) anti-predator responses are costly, so they are scaled to the level of risk, (2) anti-predator responses are additive or synergistic, so simultaneous responses are safer than either one alone, and (3) animals are naturally selected to optimize cost/benefit trade-offs of anti-predator behaviours.

3.2 Materials and Methods

Study species

Lake Sturgeon were obtained from Sustainable Sturgeon Culture, Ontario, in May 2012 where they were spawned from wild broodstock endemic to the Rainy River, Ontario. They were housed in the RJF Smith Centre for Aquatic Ecology, held in a 640 L flow-through tank and fed daily with live *Artemia* and crushed frozen bloodworm. They were maintained in dechlorinated tap water between 16-18 °C under a 14:10 hour light:dark cycle.

Stimulus preparation

I used five donor sturgeon (total length, TL: mean \pm SD = 49.95 \pm 0.11 mm) to prepare the alarm cue solution before each day of trials to control for age effects. Sturgeon were euthanized by cervical dislocation, placed in chilled dechlorinated water and homogenized using a Polytron homogenizer (Brinkman Instruments, Rexdale ON). Then, the solution was filtered through glass wool and diluted to a concentration of 25 cm² of skin \cdot L⁻¹. Solutions were frozen in 50 mL aliquots until used.

Experimental set-up

Trials took place in 100 L polyurethane D-ended mesocosms measuring 1 m long x 50 cm wide x 30 cm high (Figure 2.1). Each mesocosm was divided in half longitudinally with an opaque acrylic panel measuring 60 cm long to allow the circular flow of water with a submersible $15 \text{ L} \cdot \text{min}^{-1}$ powerhead, which provided a flow of $2\text{-}3 \text{ cm} \cdot \text{sec}^{-1}$. A rectangular section of the long-edge of the mesocosm measuring 55 cm long x 20 cm wide was segregated using vinyl-coated fiberglass mesh mounted on PVC frames. Here, four boxes measuring 20 cm long x 13.5 cm wide x 1.5 cm deep constructed from acrylic panels and sealed with silicone to hold substrates used to simulate microhabitats, were placed. A rocky microhabitat was created by layering cobble (grain size: 10-20 mm) under rocks (grain size: 30-70 mm) stacked 5 cm high. Finally, a concave ceramic tile measuring 7-10 cm long x 7.5-11.5 cm wide was imbedded in the rocks to create a large shelter. This space, together with the spaces created by the cobble and rocks, created a wide-range of interstitial zones able to accommodate fish of all sizes used in the study. A sandy microhabitat (which is an ideal foraging substrate for sturgeon) was created by filling a substrate box with sand (grain size: 0.25–1.2 mm) 1.5 cm deep. Two boxes of each microhabitat type were randomly placed in the mesocosm before the fish were introduced. The rectangular section of the mesocosm containing four microhabitat boxes, two of each type, between the mesh barriers, comprised the experimental area.

The mesocosms were filled with 25 L of water to a height of 5.5 cm over the substrate boxes to control for use of the water column during trials. Water was either left clear or turbidity was adjusted by adding 2.25 g of bentonite (an inert colloidal clay, Sigma-Aldrich; CAS number 1302-78-9) immediately before the fish were added (producing a concentration of $0.09 \text{ g} \cdot \text{L}^{-1}$, ~ 22.5 NTU, equivalent to ~17 cm secchi depth, Shoup and Wahl 2009). Turbidity measurements

were taken using a LaMotte 2020e portable turbidity meter (LaMotte Company, Chestertown MD); secchi depth measurements were taken using a 120-cm turbidity tube for intermediate turbidity levels (Wildlife Supply Company, Yulee FL). Each mesocosm was reconditioned with 1.12 g of bentonite every 6 hours to account for loss of suspended clay with time yielding an NTU range of 18.2 - 27.8 and a secchi depth range of 20 - 15 cm, respectively, during the experiment. In clear-water mesocosms, NTU was equal to 0.1. Thus, the clear- and turbid-water mesocosms provided very different physical environments for the fish during the course of the study.

Experimental protocol

Anti-predator responses were elicited with chemical alarm cues (damage-released cues) that increase the perceived level of risk in the environment where they are released (Chivers and Smith 1998; Ferrari et al. 2010). These cues are produced by a wide variety of animals including both vertebrates and invertebrates and are released following a predator attack. Exposure to alarm cues often causes prey to reduce activity and freeze or to flee from the source of the cues. Sturgeon show a very distinct increase in activity (fleeing) upon exposure to alarm cues (Wishingrad unpub. data). Alarm cue concentration, in general, has been found to be a reliable indicator of local predation risk (Ferrari et al. 2005; Brown et al. 2006; Ferrari et al. 2008). Turbidity is the analog of mist or fog in the terrestrial environment, and is believed to reduce risk by limiting the ability of visual predators to detect and/or capture prey in the aquatic environment (Lehtiniemi et al. 2005; Chiu and Abrahams 2010). The experiment was structured as a 2 x 2 factorial design with alarm cue and turbidity as main factors. Four treatment combinations resulted, and 20 replicates were run per treatment. The treatment combinations

were: (1) alarm cue/clear-water, (2) control/clear-water, (3) alarm cue/turbid-water and (4) control/turbid-water, with water injections serving as procedural controls for their respective alarm cue treatments. On each experimental day, an equal number of all treatments were run to control for behavioural variation across days. Fish were arbitrarily selected from a holding tank and three fish were placed in the experimental area of each mesocosm to provide a realistic social context and left to acclimate for 48 h prior to the onset of trials. Trials were run when the fish were between 42 days and 69 days old (28 and 55 days after the onset of exogenous feeding, respectively). Trials were conducted in June and July 2012, between 2300 hrs and 0200 hrs. Behavioural trials were conducted at night because this is the time when fish forage most actively and are therefore most susceptible to detection by predators. Incident light at an approximate intensity of 0.25 lux (equivalent to the light of a full moon on a clear night) provided a level of illumination that would likely be encountered during at least some nights in the wild. All trials were recorded using overhead cameras mounted with infrared lights, which allowed unimpaired observation of all fish from both treatments in video recordings.

All fish were fed 1 hour before the onset of trials by distributing food evenly over both substrate types to control for resource-driven habitat selection. Introducing food at this time also ensured the fish would forage actively at the initiation of trials, allowing us to directly measure trade-offs between foraging and anti-predator behaviour. Before the onset of trials, 60 mL of water from each mesocosm was withdrawn through a 2-m long injection hose and discarded (to remove stagnant water from the injection hose), then 60 mL of water from each mesocosm was withdrawn and retained (to completely flush the stimuli into the mesocosm after injection). Trials began with a 7-min pre-injection observation period (to measure baseline habitat selection and activity level), followed by a 1-min injection period of either 10 mL of alarm cue solution or 10

mL of dechlorinated tap water (control) followed immediately by 60 mL of retained water, and finally completed with a 7-minute post-injection observation period. Alarm cue was introduced at a concentration of 1 mm² of damaged skin per L in the experimental active space (Wisenden 2008) to maintain a constant level of absolute risk, and used turbid water to reduce the level of perceived risk. At the completion of trials, fish were placed in a separate holding tank and not used for subsequent trials to maintain statistical independence among trials. The experimenter was blind to treatments (alarm cue and turbidity) during trials.

Video analysis

All videos were scored using JWatcher (version 1.0; Blumstein et al. 2006). Activity level was measured by the number of times a randomly-selected focal fish passed over a superimposed 10 x 6.75 cm grid, where the side of each square was equivalent to approximately 3 to 5 body lengths of the fish. The grid pattern corresponded to transitions between each microhabitat division and a horizontal line dividing each microhabitat in half. Thus, activity level could be scored independently of transitions between microhabitats as the fish could either increase activity within one microhabitat, or alternatively, move between microhabitats. Habitat selection was measured as the proportion of total time the focal fish spent in either the sandy or rocky microhabitat. The observer was blind to cue treatment (water vs. alarm cue) during video scoring.

Statistical analysis

The data were analyzed using JMP (version 9.0.1; SAS Institute Inc. 2010). I tested for differences between treatments using 2 x 2 factorial ANOVAs to investigate the effect of risk

cue (alarm cue vs. water control) and turbidity (clear- vs. turbid-water) and their interaction on escape behaviour and changes in habitat selection. Subsequently, I analyzed differences between treatment and control groups using Tukey HSD post-hoc tests. In order to measure escape behaviour (pre-cue activity vs. post-cue escape response) I quantified escape response based on proportion change in activity level $[(\text{post} - \text{pre}) / \text{pre}]$. Five trials were excluded from analyses due to a complete lack of activity during the pre-cue injection period, yielding 19 alarm cue/clear-water, 19 control/clear-water, 18 alarm cue/turbid-water and 19 control/turbid-water replicates per treatment. Escape behaviour data were overdispersed, so I applied a rank-transformation to the proportion change in activity and analyzed the data using a 2 x 2 non-parametric ANOVA (Sheirer-Ray-Hare extension of the Kruskal-Wallis test) followed by a Tukey HSD post-hoc test. I tested baseline habitat preferences (preference for sandy vs. rocky microhabitats in the absence of risk) using a Chi-square test assuming equal expected frequency of selection for each habitat type. I quantified changes in rocky habitat use based on the difference between pre- and post-cue proportion of time spent in the rocky microhabitat (post-pre). Values for difference in proportion of time spent in the rocky microhabitats resembled a normal distribution, so I analyzed changes in habitat use using a 2 x 2 ANOVA followed by a Tukey HSD post-hoc test. I therefore base the following results for escape behaviour and cover-seeking behaviour on mean proportion change in activity and mean proportion change in time spent in rocky microhabitats, respectively.

3.3 Results

Escape response

The 2 x 2 Kruskal-Wallis H test revealed a significant interaction between alarm cue and turbidity ($H_{1,71} = 2.05, P = 0.04$). The Tukey HSD post-hoc test revealed a significant difference between the alarm cue/clear-water and control/clear-water treatments for proportion change in activity ($P < 0.001$). However, I found no significant differences in proportion changes in activity between the alarm cue/turbid-water and control/turbid-water treatments ($P = 0.15$) (Figure 3.1, upper panel).

Habitat selection and cover-seeking behaviour

In the absence of risk cues, sturgeon selected sandy microhabitats significantly more than rocky microhabitats in both clear- and turbid- environments (87.2%) ($X^2_{79} = 1153.38, p < 0.001$). The 2 x 2 ANOVA revealed a significant effect due to alarm cue ($F_{1,71} = 5.04, P < 0.001$), no effect due to turbidity ($F_{1,71} = -0.45, P = 0.66$) and no interaction between alarm cue and turbidity on the proportion of time rocky microhabitats were occupied ($F_{1,71} = 4.03, P = 0.13$) (Figure 3.1, lower panel).

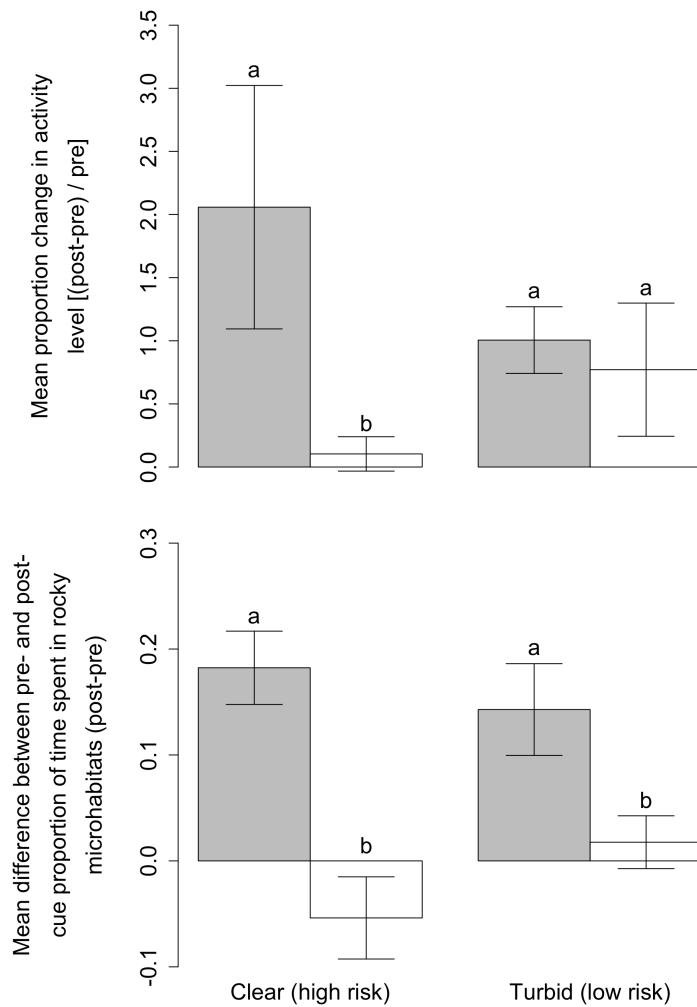


Figure 3.1. Escape response (upper panel) and changes in habitat use (lower panel) for alarm cue treatments (filled bars) and water control treatments (open bars). Alarm cue/clear-water treatments ($n = 19$) are measured against clear-water control treatments ($n = 19$), and alarm cue/turbid-water treatments ($n = 18$) are measured against turbid-water control treatments ($n = 19$). Data are means ± 1 SE. Histogram pairs sharing a lowercase letter denote statistical insignificance (i.e., $P > 0.05$).

3.4 Discussion

These results are consistent with the prediction that escape behaviours would be reduced in low-risk environments, while cover-seeking behaviour would not differ between the two treatments. In high-risk environments sturgeon elicited an escape response within rocky microhabitats that provide physical cover from predators. In low-risk environments, however, escape behaviours were reduced but cover-seeking behaviours persisted. These findings support

the idea that cover-seeking may represent a low cost/high-benefit anti-predator strategy compared to escape behaviour.

The idea that escape behaviours are costly forms the basis of many theoretical models on escape behaviour (Ydenberg and Dill 1986; Křivan 1998; Cooper and Frederick 2007) and has found empirical support as well (Skelly 1992). Likewise, the view that anti-predator responses are additive or synergistic, and simultaneous responses are better than either one alone has long been recognized. For example, Fathead Minnows (*Pimephales promelas*) are known to shoal, dash, hide, and reduce activity in response to predation (Lawrence and Smith 1989). White-tailed deer (*Odocoileus virginianus*) respond to predators by fleeing, tail-flagging (to alert conspecifics), and by snorting (to alert the predator that it has been spotted; LaGory 1987), and Black-tailed deer (*Odocoileus hemionus columbianus*) use habitat elements such as hills and vegetation in addition to fleeing from an approaching predator (Stankowich and Coss 2007). Here, I found that in high-risk clear-water environments, sturgeon responded to danger by both increasing the amount of time in rocky microhabitats and evoking a sustained escape response within these rocky areas. However, when the level of danger was reduced in turbid environments, sturgeon curtailed escape behaviours, choosing only to seek cover within rocky microhabitats in response to risk.

Seeking cover in rocky habitats is an innate behaviour in sturgeon (Peterson et al. 2007; McAdam 2011). Related sturgeon species, such as White Sturgeon (*Acipenser transmontanus*) larvae are known to use interstitial spaces between rocks as shelter from potential predators (McAdam 2011). In the present study, I used a rocky substrate (large rocks, gravel, and a ceramic shelter) to simulate a protective microhabitat because of the wide-range of interstitial spaces created are able to accommodate fish of all sizes tested throughout the study. The benefit

of protective microhabitats is presumed to be maximised when they are large enough for prey to access and small enough to exclude predators (McAdam 2011). Structural habitat heterogeneity may therefore be an important environmental feature for prey animals, allowing them to avoid energetically costly escape behaviours in predator-dominated landscapes.

Understanding the dynamics that shape predator-prey interactions is fundamentally important, especially in fish, since predation may be the single most important factor contributing to fish mortality in early life stages (Hunter 1976). Remarkably, to my knowledge, no studies to date have experimentally investigated anti-predator behaviour in Lake Sturgeon although vulnerability to predators has been investigated in White Sturgeon (Gadomski and Parsley 2005a,b,c) and Pallid Sturgeon (*Scaphirhynchus albus*) (French et al. 2010). Surprisingly, Hintz et al. (2012) were the first to document predator-avoidance behaviour in any sturgeon species. Their results complement ours in that they demonstrated that Shovelnose Sturgeon (*Scaphirhynchus platorynchus*) tended to avoid areas with predators by moving to safe areas and would flee from an attacking predator.

It is becoming increasingly important for behavioural ecologists to design studies that not only answer fundamental scientific questions, but also provide insights for conservation, protection, and management of study systems and habitats (Caro and Sherman 2011). All sturgeon species are now listed as endangered in part of their ranges, and most in all of their ranges (Billard and Lecointre 2001). Increased basic knowledge of Lake Sturgeon ecology and biology has recently emerged (reviewed in Peterson et al. 2007) but is still far from complete. For example, Lake Sturgeon preferences for sandy areas have been demonstrated in older individuals (Peake 1999), but until now have not been studied in younger fish. The finding that Lake Sturgeon appear to have a significant preference for sandy substrates in the absence of risk

is noteworthy, as anthropogenic impacts have the potential to dramatically change the relative proportion of substrate types (Maddock 1999). While sandy microhabitats appear to be important for sturgeon foraging behaviour, rocky microhabitats seem to be important for predator avoidance. Insights into how sturgeon interact with available habitats in response to predation threat is important as habitat degradation is thought to be directly responsible for declines in sturgeon abundance (Birstien 1993; Auer 1996). Based on the results presented here, habitat conservation and restoration efforts should consider preserving sandy and rocky microhabitats, as they are both important for sturgeon life-history. Rocky microhabitats may be especially important to conserve or restore in areas where predation pressure is particularly high. Across taxonomic groups, habitat heterogeneity is often correlated with local biodiversity owing to more niche diversity within heterogeneous habitats (Tews et al. 2004). Furthermore, animals often mediate predation risk by movement and habitat selection across heterogeneous landscapes (Kauffman et al. 2007). Structural habitat heterogeneity may promote local biodiversity by allowing prey animals to elicit low-cost/high-benefit anti-predator behaviours when faced with predators, increasing their probability of survival. A low-cost/high-benefit anti-predator strategy, such as cover-seeking behaviour may be vitally important for the preservation of this and other similarly endangered species.

Overall, this study strengthens our understanding of the relative cost/benefit trade-off of two common anti-predator behaviours. However, important insights could be gained from future studies that test the benefit of escape behaviours and changes in habitat use directly in the face of different predators or environmental constraints. Investigating how other species behave in similar contexts would be useful for finding patterns or differences between and among taxa.

Continuing to explore how animals use environmental features in the context of predation should continue to be an exciting area of research.

CHAPTER 4: BEHAVIOURAL AND MORPHOLOGICAL DEFENSES IN A FISH WITH A COMPLEX ANTI-PREDATOR PHENOTYPE

4.1 Introduction

Predation is a strong selective force shaping the lives of prey animals. Not only can predators reduce prey numbers through direct consumption, they cause non-consumptive effects influencing an animal's morphology, life history, and behaviour including when, where and how they forage and reproduce (Preisser et al. 2005). An animal's success in avoiding predators involves an integration of multiple defences, so there should be strong selection maintaining adaptive trait combinations in natural populations (Sinervo and Svensson 2002).

Perhaps the most well-known example of correlational selection between anti-predator behaviour and morphology is that of garter snake (*Thamnophis ordinoides*) escape behaviour and the pattern on their back (Brodie 1989; Brodie 1992). Snakes with striped back-patterns that fled in straight lines, and garter snakes with barred back-patterns that fled while reversing direction, each had significantly higher survival rates in the wild compared with barred snakes that fled in straight lines, and striped snakes that fled while reversing direction (Brodie 1992). In this case, specific body pattern and behaviour combinations that increase survival led to genetic correlations between morphology and anti-predator behaviour (Brodie 1989).

Dewitt et al. (1999) proposed a framework for understanding how anti-predator traits interact. Mechanically independent traits can interact in an additive way (trait co-specialization), one trait can compensate for another (trait compensation), or traits may need to be combined in order to be efficient (trait complementation) and finally, mechanically linked traits may rely on each-other to function (trait co-dependence). In an influential paper, Dewitt et al. (1999)

provided evidence of trait compensation and co-specialization in a freshwater snail, and sparked a great deal of research exploring how correlated traits evolve. This can be important in helping us understand the fitness consequences of behaviour and the evolution of integrated phenotypes.

Differences in morphology can influence how individuals within a species behave. For example, Pettersson et al. (2000) found that Crucian Carp (*Carassius carassius*) from populations that coexist with predators—and as a consequence developed deep bodies, exhibit weaker fright responses (i.e., trait compensation). Likewise, deep-bodied Goldfish (*Carassius auratus*) exhibited a reduced response to predator odours than shallow-bodied conspecifics (Chivers et al. 2007). Moreover, both shallow and deep-bodied fish responded more strongly to predators fed conspecifics with the same morphology (Chivers et al. 2007).

Differences in morphology between species may also correlate with differences in anti-predator behaviour. Abrahams (1995) found that Brook Stickleback (*Culea inconstans*), which have a series of spines on their dorsal surface, showed little behavioural modification in response to a predator, while unarmoured Fathead Minnows (*Pimephales promelas*) responded significantly more. Similarly, Rundle and Brönmark (2001) found evidence of species-specific patterns of trait compensation in predator-defense adaptations across several species of pulmonate snails. These studies provide evidence that morphological differences between species are associated with predictable differences in anti-predator behaviour. However, behaviour of distantly related species used in these types of comparative studies may be the result of differences in life-history or body shape, and may obscure the true relationship between morphology and behaviour (Mikolajewski and Johansson 2004). In order to help address this problem, Mikolajewski and Johansson (2004) investigated the relationship between behaviour and morphological defense in larvae of three dragonfly species within the genus *Leucorrhinia*.

They found that the three different species exhibited a combination of trait compensation and co-specialization. One potential confounding factor, however, could be differences in predator abundance between lakes where dragonfly larvae were collected (Mikolajewski and Johansson 2004).

Understanding the interplay between morphology and behaviour continues to be an exciting area of research. However one major deficit in our understanding of how behaviour and morphology interact is that it has so far been limited to somewhat simple systems—mostly having to do with various properties of snail shell morphology, body size or armour in fish, and spine length in dragonfly larvae. Furthermore, how behaviour changes alongside the development of defensive morphological adaptations in the absence of potential confounding effects of predator exposure is not well understood. Finally, the relative degree to which several complementary morphological traits co-vary with anti-predator behaviour has not been examined. Lake Sturgeon (*Acipenser fulvescens*) is an ideal species in which to investigate these questions. Anti-predator behaviours include increased activity (an escape response) and increased residency in rocky habitats (cover-seeking). Anti-predator morphological adaptations consist of five rows of scutes (armour/spines), a mottled (cryptic) body pattern that develops in juveniles, and fast-growing bodies. Furthermore, Lake Sturgeon is an ideal species in which to investigate how behaviour changes alongside morphological development because they undergo a dramatic morphological change in a relatively short time period. The extent and rate at which these morphological changes proceed is so dramatic it is almost like metamorphosis (Peterson et al. 2007). Finally, since Lake Sturgeon possess several different morphological defense traits, unravelling the degree to which anti-predator behaviours co-vary with variable morphology may be possible.

In the present study I investigate how escape and cover seeking behaviours vary in response to changes in several independent morphological traits. I also investigate how these two anti-predator responses change as morphological development proceeds. I predict that as these morphological defenses develop, anti-predator behaviour will decrease (i.e., trait compensation) because this is the most common way behavioural and morphological traits interact (Mikolajewski and Johansson 2004). I also investigate the hierarchical structure of behavioural dependence on morphology (i.e., which morphological adaptations co-vary most strongly with anti-predator behaviour), but given the lack of available data, I am unable to make any reasonable *a priori* predictions about this outcome, so I approach this component in a purely exploratory way.

4.2 Materials and Methods

Study species

Lake Sturgeon were obtained from Sustainable Sturgeon Culture, Ontario, in May 2012 where they were spawned from wild broodstock endemic to the Rainy River, Ontario. They were housed in the RJF Smith Centre for Aquatic Ecology, held in a 640 L flow-through tank and fed daily with live *Artemia* and crushed frozen Chironomidae. They were maintained in dechlorinated tap water between 16-18 °C under a 14:10 hour light:dark cycle.

Experimental set-up

Trials took place in 100 L polyurethane D-ended mesocosms measuring 1 m long x 50 cm wide x 30 cm high (Figure 2.1). Each mesocosm was divided in half longitudinally with an opaque acrylic panel measuring 60 cm long to allow the circular flow of water with a

submersible $15 \text{ L} \cdot \text{min}^{-1}$ powerhead, which provided a flow of $2\text{-}3 \text{ cm} \cdot \text{sec}^{-1}$. A rectangular section of the long-edge of the mesocosm measuring 55 cm long x 20 cm wide was segregated using vinyl-coated fiberglass mesh mounted on PVC frames. Each mesocosm contained sandy microhabitats (which is an ideal foraging substrate for sturgeon), and rocky microhabitats (which provide physical shelter). A rocky microhabitat was created by layering cobble (grain size: $10\text{-}20 \text{ mm}$) under rocks (grain size: $30\text{-}70 \text{ mm}$) stacked 5 cm high. Also, a concave ceramic tile measuring $7\text{-}10 \text{ cm}$ long x $7.5\text{-}11.5 \text{ cm}$ wide was imbedded in the rocks to create a large shelter. This space, together with the spaces created by the cobble and rocks, created a wide-range of interstitial zones able to accommodate fish of all sizes used in the study, enabling sturgeon to effectively seek cover in rocky microhabitats. A sandy microhabitat was created by filling a substrate box with sand (grain size: $0.25\text{-}1.2 \text{ mm}$) 1.5 cm deep. These microhabitats were constructed in boxes measuring 20 cm long x 13.5 cm wide x 1.5 cm deep. Two boxes of each microhabitat type were randomly placed in each mesocosm before the fish were introduced. The mesocosms were filled with 25 L of water to a height of 5.5 cm over the substrate boxes to control for use of the water column during trials. The rectangular section of the mesocosm containing four microhabitat boxes, two of each type, between the mesh barriers, comprised the experimental area.

Stimulus preparation

Anti-predator responses were elicited with alarm cues (\sim conspecific damage-released cues) that increase the perceived level of danger in the environment where they are released (Chivers and Smith 1998; Ferrari et al. 2010). Five donor sturgeon were used to prepare the alarm cue solution before each day trials were conducted. Sturgeon were euthanized by cervical

dislocation followed by a concussive blow to the head, and then were placed in chilled dechlorinated water and homogenized using a Polytron homogenizer (Brinkman Instruments, Rexdale ON). Then, the solution was filtered through glass wool and diluted to a concentration of $25 \text{ cm}^2 \text{ of skin} \cdot \text{L}^{-1}$. Solutions were frozen in 50 mL aliquots until used. Sturgeon could not be anesthetised prior to euthanizing them, because of the potential contamination of the alarm cue solution with the anaesthetic.

Experimental protocol

Trials took place in June and July 2012 when sturgeon were between 42 and 69 days-post-hatch (dph), and again in September and October 2012 when the fish were between 130 and 148 dph. A total of 80 trials were run between June and July and between September and October (20 alarm cue and 20 water control in each of the two groups). Fish were arbitrarily selected from a holding tank and three fish were placed in the experimental area of each tank and left to acclimate for 48 h prior to the onset of trials. Trials were conducted between 2300 and 0200 hrs. Trials were conducted at night because this is the time when fish forage most actively and are therefore susceptible to detection by predators. Incident light at an approximate intensity of 0.25 lux (equivalent to the light of a full moon on a clear night) provided a level of illumination that would likely be encountered during at least some nights in the wild. A small amount of crushed bloodworm was added to each mesocosm 1 hour prior to behavioural observations. Introducing food to the mesocosms at this time guaranteed the fish would forage actively at the onset of trials so trade-offs between foraging and anti-predator behaviour could be assessed. All trials were recorded using overhead cameras mounted with infrared lights. Before the onset of trials, 60 mL of water from each mesocosm was withdrawn through a 2-m long

injection hose and discarded (to remove stagnant water from the injection hose), then 60 mL of water from each mesocosm was withdrawn and retained (to completely flush the stimuli into the mesocosm after injection). Trials began with a 7-min pre-injection observation period (to measure baseline habitat selection and foraging activity level), followed by a 1-min injection period of either 10 mL of alarm cue solution or 10 mL of dechlorinated tap water (control) followed immediately by 60 mL of retained water, and finally completed with a 7-minute post-injection observation period. Alarm cue trials were conducted to examine how Lake Sturgeon respond to risk, while water control trials were conducted to verify that the observed behavioural responses were due to risk cues rather than the injection *per se*. The experimenter was blind to cue treatment (water vs. alarm cue) during injections. Alarm cue was introduced at a concentration of 1 mm² of damaged skin per L in the experimental active space (Wisenden 2008) to maintain a constant level of absolute risk. At the completion of trials, focal fish whose behaviour was scored were photographed for morphometric analysis. After photographs were taken, all fish were placed in a separate holding tank and not used for subsequent trials to maintain statistical independence among trials.

Video analysis

All videos were scored using JWatcher (version 1.0; Blumstein et al. 2006). Activity level was measured by the number of times a randomly-selected focal fish passed over a superimposed 10 x 6.75 cm grid, where the side of each square was equivalent to approximately 3 to 5 body lengths of the fish. The grid pattern corresponded to transitions between each microhabitat division and a horizontal line dividing each microhabitat in half. Thus, activity level could be scored independently of transitions between microhabitats as the fish could either

increase activity within one microhabitat, or alternatively, move between microhabitats. Rocky microhabitat occupancy was measured as the proportion of total time the focal fish spent in the rocky microhabitat. The behaviour of the other two fish was not scored—they were added to provide a realistic social context during the experiment as juvenile Lake Sturgeon are often found in groups. The observer was blind to cue treatment (water vs. alarm cue) during video scoring.

Morphometric analysis

Dorsal and lateral photographs were taken of each focal fish using a digital camera (Panasonic DMC-FZ28; ~50 pixels/mm on image sensor). Photographs were scored using ImageJ (version 1.45s; Schneider et al. 2012). Fish length, width, and height were measured to estimate body size, and the height of each dorsal scute was measured to estimate scute size. All measurements were made to the closest 0.1 mm; measurements at this scale were very highly repeatable ($r > 0.9$). Mottling was absent in the young group and present in the older group. Photographs of representative fish are provided in Figure 4.1 and descriptive statistics for morphometric data are presented in Table 4.1.

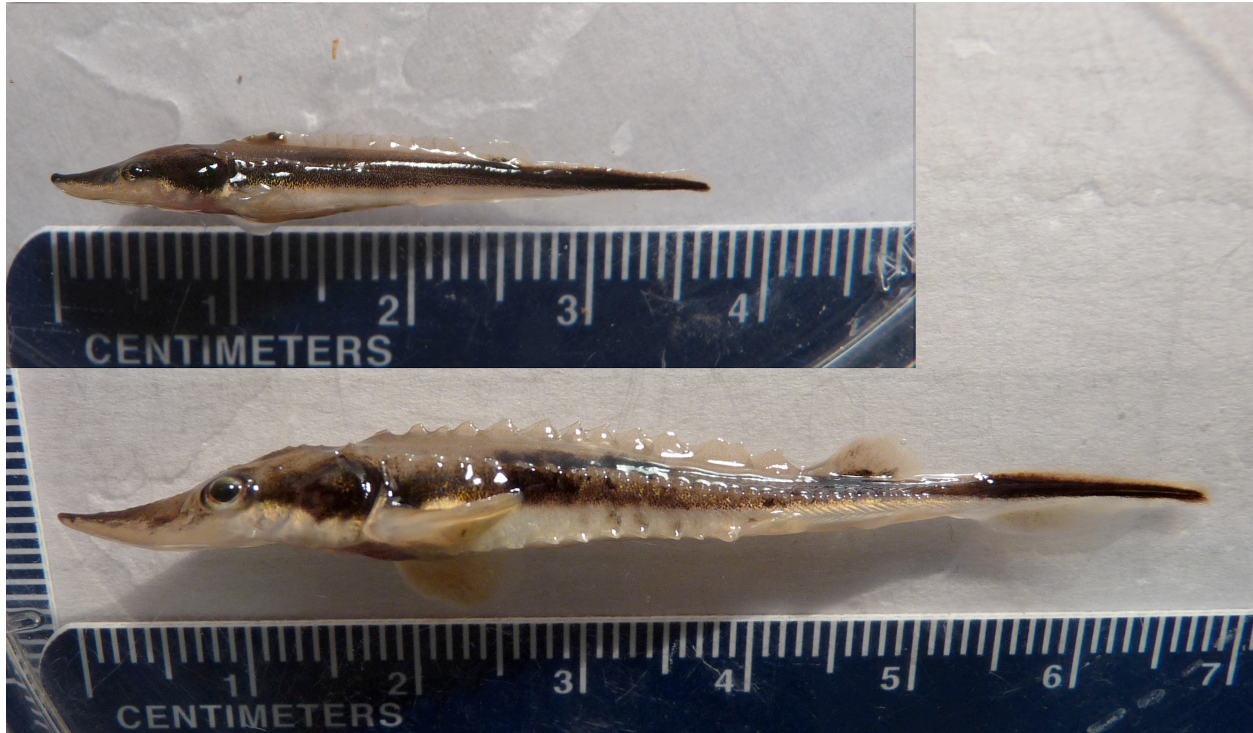


Figure 4.1 Lateral images of Lake Sturgeon showing the range in size, scute development, and change in colour pattern over the course of the study. The small fish (above) is 45 days old; the large fish (below) is 148 days old.

Table 4.1 Morphometrics of Lake Sturgeon used in the study. The sturgeon are divided into two groups: young fish (that lack mottled colouration) and older fish (with mottled body patterns). Length is measured as total length (tip of rostrum to tip of tail). Width and height are measured immediately anterior to pectoral fins. Scutes is an average height of all dorsal scutes. Units are in millimeters. Sample size is 40 per group.

		Length	Width	Height	Scutes
Mottling absent (42 - 69 dph)	Mean	40.9	4.1	4.0	0.7
	Std. Dev.	6.8	0.7	0.7	0.2
Mottling present (130 -148 dph)	Mean	67.0	8.7	7.1	1.2
	Std. Dev.	6.7	1.0	0.8	0.2

Statistical analysis

The data were analyzed using R (version 2.15.2; R Core Team 2012). Escape responses were defined as the relative change in line crosses ($[\text{post-pre}]/[\text{pre}]$). The escape response

distribution was \log_e+1 transformed prior to analysis to achieve normality. This transformation was chosen because the distribution was positively skewed, and the minimum value was -0.75. Cover-seeking behaviour was defined as the inverse relative change in proportion of time spent in sand microhabitats ($[\text{post-pre}]/[\text{pre}]$). The distribution of cover-seeking behaviour was normally distributed and not transformed prior to analysis.

Principal Components Analysis (PCA) was used to reduce dimensionality in the body size variables to generate a 'body size' composite variable from the length, width, and height measurements (Jolliffe 2005). Similarly, average dorsal scute height was calculated to generate a single 'scute size' variable to be used in the analysis. Pearson's test for significant correlations was used to investigate if escape responses and cover use in response to alarm cues were significantly correlated. Regression tree analysis was used to investigate how each anti-predator behaviour (escape behaviour and cover-seeking) co-varied with different morphologies using the *rpart* package (Therneau et al. 2012). Regression tree analysis produces a predictive tree-like diagram based on recursive partitioning of variance in the response data, and gives an indication of the influence of the explanatory variables at each split (node) in the regression tree (De'ath and Fabricius 2000; Legendre and Legendre 2012). The dependent variable was either escape response or cover-seeking, and the independent variables were body size, scute size (average of all dorsal scutes), and mottling (present or absent). Cross-validation was used to select tree size by selecting trees with the minimum cross-validated standard error (De'ath and Fabricius 2000).

4.3 RESULTS

Behavioural correlations

Escape behaviour and cover seeking in response to alarm cues were not significantly correlated ($r = 0.26$, $N = 40$, $P = 0.1$), so each anti-predator behaviour was analyzed separately to investigate how morphological traits contribute uniquely to each response.

Body size PCA

PC axis 1 (i.e., ‘body size’ index) explained 99.7% of the variation in body size measurements. Body size was very significantly positively correlated with length, width and height (all $r > 0.95$, $N = 40$, $P < 0.001$). Length was therefore used instead of body size index in the subsequent analysis to simplify the interpretation of the results.

Regression trees

Regression tree results where length was used as a predictor variable are presented. In all cases the results obtained using body size were identical to those obtained using length (i.e., cross validation selected the same number of groups). Length was chosen in the analysis because the regression trees would identify specific threshold values, which are easier to interpret as a length rather than the body size index of three morphological variables. Additionally, the use of length instead of body size allows for an easier comparison between other studies and systems.

The escape response regression tree is presented in Figure 4.2. Fish length was the only morphological variable that predicted escape responses, where counterintuitively, larger fish exhibited a stronger response than smaller fish (average percent increase in activity of 843% vs. 215%).

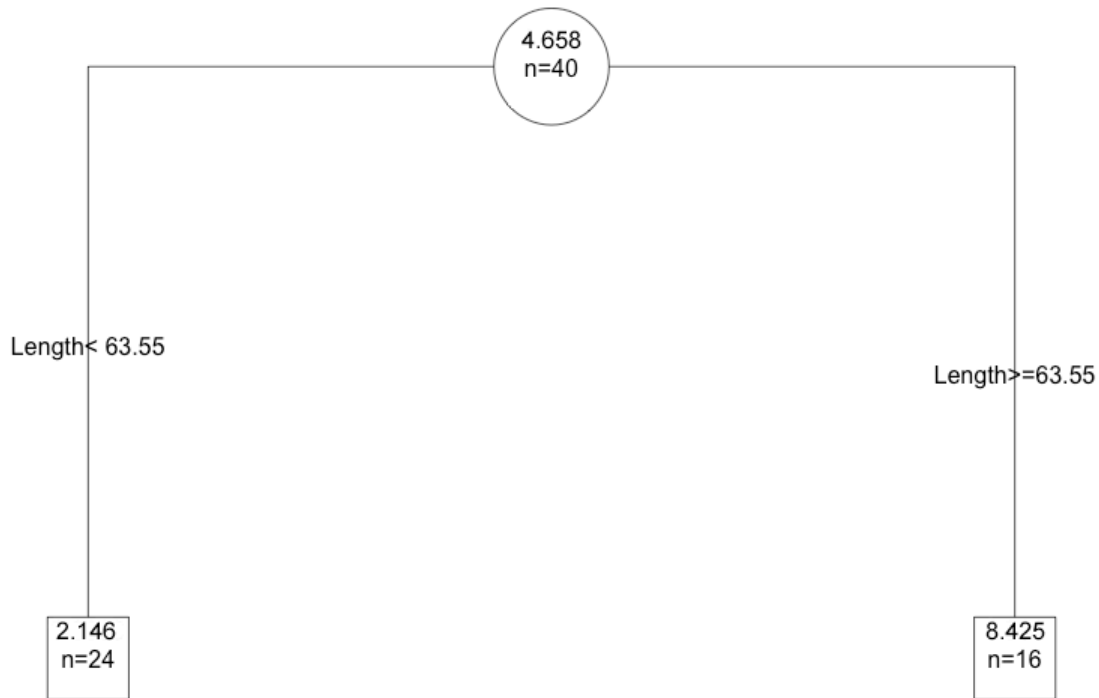


Figure 4.2 Regression tree showing the relationship between escape behaviour (increased activity) relative to the three morphological variables (body size, scute size, body pattern) based on 40 trials. Values at each node indicate the mean proportion change in activity level following exposure to alarm cues. Values along each branch indicate the mean fish length in mm. This tree was pruned from a 4-level tree to a 1-level tree based on cross-validation.

The cover-seeking regression tree is presented in Figure 4.3. The smallest fish in the study increased the percentage of time spent in rocky areas the most (~32% increase) after exposure to alarm cues relative to the pre-stimulus baseline. The largest fish in the study also increased the percentage of time they spent in rocky areas (~26% increase), but less so than the smallest fish. Mottled fish of intermediate size showed a small increase in the proportion of time spent in rocky areas (~3.1%) relative to other fish of equivalent size that lacked the mottled body pattern (increase of ~12%) after exposure to alarm cues.

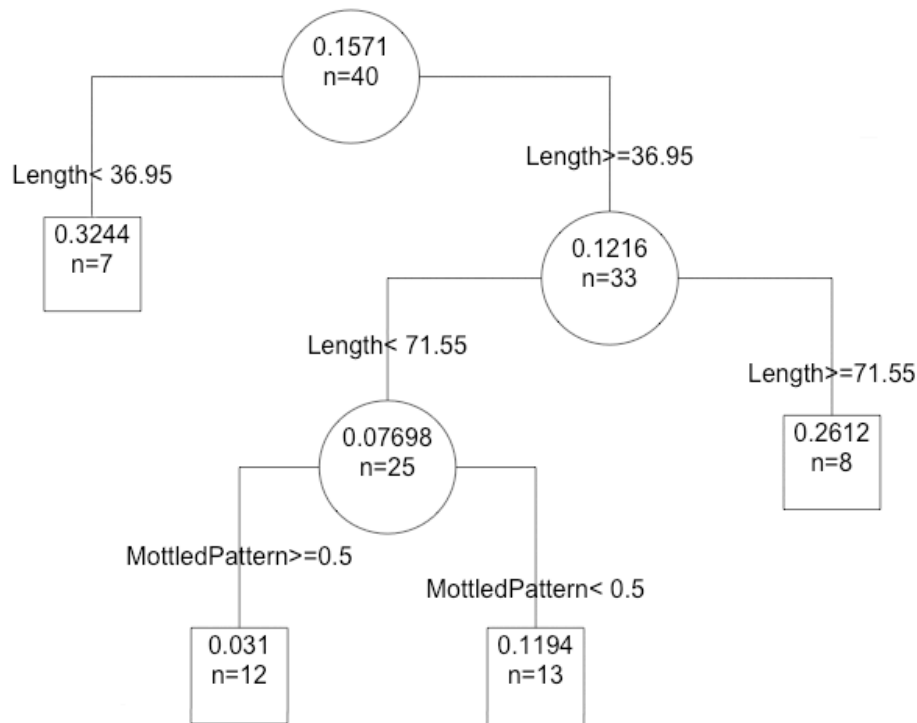


Figure 4.3 Regression tree showing the relationship between cover-seeking behaviour (increased time in rock microhabitats) relative to three morphological variables (body size, scute size, body pattern) based on 40 trials. Values at each node indicate the mean proportion increase in time spent in rocky areas following exposure to alarm cues. Values along the upper branches indicate the mean fish length in mm. Values along the lowermost branches indicate the presence of mottled body pattern (≥ 0.5) or absence of mottled body pattern (< 0.5) (i.e., coded as binary). This tree was pruned from a 4-level tree to a 3-level tree based on cross-validation.

Anti-predator behaviour between stages and treatments

Table 4.2 compares the responses of sturgeon to alarm cues at two stages of development. Overall, larger fish elicited stronger escape behaviours in response to alarm cues than smaller fish. On the other hand, larger fish spent less time in rocky microhabitats in response to alarm cues than smaller fish. Anti-predator responses to water injections were within 1 SE of zero in all treatment combinations (except for one case in which the response was weakly negative), verifying that the observed anti-predator responses are in fact due to alarm cues.

Table 4.2 Descriptive statistics for escape and cover-seeking anti-predator behaviors comparing responses of young fish (that lack mottled colouration) and older fish (that have mottled body patterns). Escape behaviour is relative change in activity (relative to the pre-stimulus baseline) and calculated using the formula $([post-pre]/[pre])$. Cover-seeking behaviour is the inverse relative change in proportion of time spent in sand microhabitats. Filtered dechlorinated tap water was used as a control.

		Escape response		Cover-seeking	
		Alarm Cue (n=20)	Control (n=20)	Alarm Cue (n=20)	Control (n=20)
Mottling absent (42 - 69 dph)	Mean	1.96	0.12	0.19	-0.11
	Std. Err	0.92	0.13	0.04	0.07
Mottling present (130 -148 dph)	Mean	7.35	0.08	0.12	-0.01
	Std. Error	2.42	0.26	0.04	0.02

4.4 DISCUSSION

I found strong evidence for trait co-dependence between escape responses and body size. It is easy to predict that the relationship between body size and escape responses would be inversely related (trait compensation), because increased body size may make sturgeon less vulnerable to predators and allow them to reduce their investment in costly anti-predator behaviour. However, the relationship I found suggests that the magnitude of escape responses is mechanistically linked to body size. As sturgeon grow larger, their tailfin area increases, accessory fins grow larger, and swimming musculature becomes more highly developed—all of which allow sturgeon to evoke stronger and more sustained escape responses. Indeed, Lake Sturgeon swimming endurance has been found to increase with length (Peake et al. 1997), and in a closely related species, White Sturgeon (*Acipenser transmontanus*), small juveniles (< 82 mm TL) had lower escape speeds than medium (82-92 mm TL) and large (> 93 mm TL) juveniles (Boysen and Hoover 2009). Furthermore, the results obtained here suggesting that escape

responses and body size are co-dependent are corroborated by what we know about the evolution of Actinopterygian morphology. Specifically, high power caudal propulsion is believed to have evolved due to selective pressure from pursuit predators (Webb 1982).

Cover-seeking behaviour exhibited a complex multi-tiered relationship that depends on specific morphological combinations. Similar to the results for escape responses, body size was the primary morphological trait linked to cover-use. However, the relationship was inversely related to that of escape response. The smallest fish in the study occupied rocky microhabitats more than any other size class, indicating that occupying protective rocky microhabitats may compensate for small body size. This result matches my predicted hypothesis concerning the relationship between body size and cover-seeking behaviour. However, the largest fish in the study also increased the amount of time spent in rocky areas, although not to the same degree as the smallest fish. This might be evidence of trait co-specialization. The largest fish tested here may be able to maneuver through rocky microhabitats quickly and swiftly and be able to use rocky features to their advantage when faced with a pursuit predator, further increasing their probability of survival, but I presently have no data to support this hypothesis. Finally, mottled sturgeon of intermediate size did not increase the amount of time they spent in rocky areas, whereas sturgeon that lacked this cryptic colour pattern did increase the amount of time spent in rocky areas. The former case may represent a form of trait compensation, allowing mottled sturgeon to remain in foraging grounds even in riskier situations. The latter case may also represent a case of trait compensation, albeit of a different sort. Here, sturgeon that lacked the mottled body pattern appear to compensate by seeking cover in rocky areas. In this case, selection appears to have favoured specific behaviours that are dependent on the presence or absence of cryptic colouration. This is not surprising given the fact that several previous studies

have found that predators can impose correlational selection on prey colour pattern and behaviour (Brodie 1989; Forsman and Appelqvist 1998).

A surprising outcome of this study is that scute size did not co-vary whatsoever with sturgeon behaviour. Scutes are energetically costly to produce and increase energetic costs when swimming due to increased drag (Peterson et al. 2007). I therefore predicted that this cost would be offset by a reduction in anti-predator behaviour (i.e., trait compensation). However, it may be that at this stage of development sturgeon scutes are not large enough or sharp enough to provide an effective defense against predators, which would prevent behavioural changes in response to scute development from evolving in sturgeon during this life-stage. As sturgeon mature and their scutes grow larger and stronger, any behavioural effects due to scutes may become evident. On the other hand, it may be that scutes simply co-specialize with anti-predator behaviour in sturgeon to maximize survival. Further study is clearly needed to resolve this issue.

Together, these results suggest that behaviour co-varies most strongly with body size, followed by cryptic colouration, which in turn co-varies with behaviour more than defensive morphological structures (scutes) at this stage of development. Additionally, I found that body size may be co-dependent, compensatory, and perhaps even co-specialized as well across different stages of morphogenesis. Indeed, selection acts on total organism performance, and traits (and interactions between traits) need not be limited to a single mechanism so long as there is adaptive value to the decision rule directing and adjusting behaviour.

Evaluating the adaptive value of these behaviour-morphology combinations directly would require an empirical test of their survival value in natural populations. However, all sturgeon species are now listed as endangered in part of their ranges, and most in all of their ranges (Birstein et al. 1997; Billard and Lecointre 2001). Additionally, sturgeon are more

critically endangered than any other group of species on the planet today (IUCN 2010), precluding the possibility of testing the survival value of behavioural-morphology trait combinations at this time. If and when sturgeon populations recover, testing the adaptive value of these integrated traits is an obvious next step.

Frequency-dependent biotic interactions, such as predator-prey relationships, are often regarded as strong enough to produce and maintain adaptive genetic correlations among fitness-related traits (Sinervo and Svensson 2002). As sturgeon morphology has been largely preserved since the lower Jurassic period (~200 million years ago) it may represent a highly successful morphological trait complex to which behaviour is finely tuned. This study sheds light on how behaviour interacts with morphology in a dynamic multi-trait system. Insights into the extent to which behaviour co-varies with morphological development is important in order to understand the limitations or reaches of adaptive phenotypic plasticity and the evolution of integrated phenotypes. Continuing to understand the manner by which selection acts on integrated phenotypes should prove to be an exciting area of future research.

CHAPTER 5: GENERAL DISCUSSION

Lake Sturgeon anti-predator behaviour is incredibly complex, and is mediated by water clarity, habitat structure, and defensive morphology.

5.1 Predation Risk and Environmental Change

I found that water clarity alone can exert strong effects on sturgeon anti-predator behaviour. This change in behaviour may be mediated through non-lethal predation effects, where the probability of being detected by a predator can have a significant effect on prey behaviour (Lima 1998). Increased turbidity (decreased water clarity) seems to reduce the need for Lake Sturgeon to remain motionless to avoid detection by visual predators. However, decreased turbidity (increased water clarity) seems to increase Lake Sturgeon anti-predator inactivity and therefore significantly reduces their foraging behaviour. However, Lake Sturgeon did not hide in interstitial spaces between rocks during daylight periods, preferring instead to rest motionless on the surface of sandy substrates.

In other animal species, environmental variables that correlate with predator abundance or performance may have similar effects. For example, in cases where predator abundance or performance is driven by temperature, salinity, or pH, prey may adjust their behaviour in response to these environmental cues to avoid potential encounters with predators. Future work aimed at understanding how environmental changes affect predation risk will help us understand the effect of environmental changes on behaviors shaped and driven by predation. Even now the degree to which behaviourally plastic responses are influenced by changing environmental cues is not well understood. Given that rapidly changing climates and habitats represent important

challenges faced by the majority of animal species, and are leading to rapid declines in global biodiversity, they deserve more attention.

5.2 Anti-predator Behaviour and Structural Habitat Heterogeneity

While rocky habitats do not seem to be important for Lake Sturgeon anti-predator behaviour during the course of the diel cycle, or between clear- or turbid-water environments (i.e., as probability of risk changes), rocky habitats seem to be very important for Lake Sturgeon anti-predator behaviour in response to chemical alarm cues (i.e., as actual level of risk changes). In clear water (high risk environments) Lake Sturgeon significantly increased activity levels (an escape response) and significantly increased the proportion of time spent in rock microhabitats (cover seeking behaviour). Lake Sturgeon seem to evoke both behaviours simultaneously in response to high risk by swimming quickly *within* rocky areas. Furthermore, rocky microhabitats may be especially important for Lake Sturgeon anti-predator behaviour because in turbid water (low risk environments) Lake Sturgeon significantly increased the proportion of time spent in rocky microhabitats, but did not flee to a significant degree. Therefore, the cost/benefit trade-off may be lower for cover-seeking behaviour than for escape behaviour. This finding highlights the importance structural habitat heterogeneity may play in enabling Lake Sturgeon to elicit a low cost/ high-benefit anti-predator behaviour.

Across the animal kingdom, prey are known to flee from predators and seek shelter in protective habitats. It has long been known that escape behaviours are costly (Ydenberg and Dill 1986; Skelly 1992; Křivan 1998; Cooper and Frederick 2007). Structural habitat heterogeneity may therefore be an important feature of the environment, especially in predator-dominated landscapes, allowing prey animals to elicit a low-cost/high-benefit anti-predator behaviour.

5.3 Morphology and Anti-predator Behaviour

Morphology seems to be an important mediator of risk for Lake Sturgeon. Since predation risk on Lake Sturgeon is largely size dependent (Peterson et al. 2007), it is easy to predict that as they grow larger their anti-predator behaviour would decrease. However, as Lake Sturgeon grew larger they evoked stronger escape behaviours in response to alarm cues. The magnitude of escape behaviour seems to be tightly linked with body size, where larger sturgeon are able to flee more quickly and for a longer period of time. I would, however, expect sturgeon escape responses to decrease substantially when they outgrow local predators. Cover-seeking behaviour was also tightly linked to morphology. The smallest fish tested increased the amount of time in rocky areas more than fish of any other size. Interestingly, whether fish of intermediate size moved into rocky areas or not depended on their body colouration: fish lacking cryptic colouration increased their proportion of time in rocky areas much more than cryptic (mottled) fish. These two findings highlight the sophistication of Lake Sturgeon anti-predator behaviour and its link of their morphology.

Frequency-dependent biotic interactions, such as predator-prey relationships, are often regarded as being strong enough to produce and maintain adaptive genetic correlations among fitness-related traits (Sinervo and Svensson 2002). As sturgeon morphology has been largely preserved since the lower Jurassic period (~200 million years ago) it may represent a highly successful morphological trait complex to which behaviours are finely tuned. Insights into the extent to which behaviour varies in response to morphological development is important in order to understand the limitations or reaches of adaptive phenotypic plasticity and the evolution of

integrated phenotypes. Continuing to understand the manner by which selection acts on integrated phenotypes should prove to be an exciting area of future research.

5.4 Conservation Notes

It is becoming increasingly important for behavioural ecologists to design studies that not only answer fundamental scientific questions, but also provide insights for conservation, protection, and management of study systems and habitats (Caro and Sherman 2011). All sturgeon species are now listed as endangered in part of their ranges, and most in all of their ranges (Billard and Lecointre 2001). Two main conclusions, which have implications for sturgeon conservation, can be drawn from the results presented in this thesis.

First, changing turbidity levels are likely to influence behaviour of young sturgeon and may have fitness consequences. Turbidity, therefore, should be considered in habitat restoration programs. Increased turbidity is likely to be highly beneficial to young sturgeon, whether predators are present or not. Clear water, on the other hand, may significantly restrict foraging activity in young sturgeon, even in the absence of predators.

Second, sandy microhabitats may be important for sturgeon foraging behaviour and rocky microhabitats may be important for sturgeon predator avoidance. Habitat conservation and restoration efforts should consider preserving sandy and rocky microhabitats, as they both seem important for sturgeon life-history. Rocky microhabitats may be especially important to conserve or restore in areas where predation pressure is particularly high.

However, more research is needed to understand the role of turbidity and habitat structure on sturgeon foraging at later life stages. Even if increased turbidity and rocky microhabitat accessibility increase survival in young sturgeon, it may have a negative impact on adult

sturgeon foraging success if, for example, prey utilize rocky microhabitats to escape predation by adult sturgeon. Conservation management plans should strive to maximize lifetime reproductive success of the target organism, rather than simply focusing on survival at a specific life stage. Furthermore, it is important to recognize the wide-range of trade-offs in conservation biology, and management actions that may improve survival for one species may have detrimental effects on other species.

5.5 Final Thoughts

Lake Sturgeon is an interesting and unique species, and while its behaviour and ecology is not well understood, it is my hope that this thesis becomes a foundation for future research investigating the behavioural ecology of this species.

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